RESEARCH ARTICLE

Changes in resistance pattern of ESKAPE pathogens between 2010 and 2020 in the clinical center of University of Szeged, Hungary

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ABSTRACT

The acronym ESKAPE stands for six antibiotic-resistant bacterial pathogens namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. Monitoring their resistance is an important task for clinical microbiology laboratories.

Our aim was to analyze the resistance patterns of these bacteria over ten years in clinical samples of our department. We examined the sample types from which these pathogens were most frequently isolated. The incidence of tests with resistant results for each pathogen in aggregate and the most important subgroups of each was also analyzed. We have also intended to predict the local priorities amongst these pathogens.

The results of 1,268,126 antibiotic susceptibility tests performed on a total of 70,099 isolates over this period were examined. Most strains were derived from urine, blood culture, trachea, vagina, wounds, and abscesses. Prevalence of ESKAPE bacteria increased between 2011 and 2020 however, the steepest intensifications were seen in the cases of *K. pneumoniae* and *P. aeruginosa*. The number of antibiotic susceptibility tests with resistant results has also increased over the decade but the most notable increase was detected in *E. faecium* and *A. baumannii*. Based on the calculation of antimicrobial resistance index for each pathogen, the most serious challenges for us at present are *A. baumannii*, *P. aeruginosa*, and *E. faecium* and their multi-resistant forms.

The theoretical prediction of proportion of resistant tests between 2020 and 2030 in our care area draws attention to a worrying trend in the cases of vancomycin-resistant *E. faecium* and carbapenem-resistant *A. baumannii* strains.

INTRODUCTION

The acronym ESKAPE stands for six highly virulent and antibiotic-resistant bacterial pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species [1]. These bacteria are capable of 'escaping' the biocidal action of antibiotics and jointly represent new paradigms in transmission and resistance [2]. Thus, ESKAPE bacteria embody the most challenging kind of nosocomial pathogens, because of their high-level antimicrobial

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resistance [1]. In 2017, the World Health Organisation published its list of pathogenic bacteria for which new anti-microbial development is urgently needed, on which the ESKAPE pathogens were designated priority status [3]. Antibiotic resistance in ESKAPE organisms is usually associated with significantly higher morbidity, mortality, as well as an economic burden [4]. In Europe, over 33,000 deaths and 874,000 disability-adjusted life-years are attributed to hospital-acquired and community-acquired antibiotic-resistant infections, including ESKAPE each year [5]. Thus, monitoring the antibiotic resistance of ESKAPE pathogens is an important task for clinical microbiology laboratories all over the world.

In the European Union, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is responsible for harmonizing and standardizing antimicrobial susceptibility testing [6, 7]. In Hungary, antimicrobial susceptibility testing according to these standards has been carried out nationwide since 2012. This makes our data internationally comparable.

The ever-increasing problem of resistance requires ever more advanced solutions. One way of doing this is to analyze trends and, on this basis, to predict the evolution of resistance rates [8]. Knowledge of these expected trends can form the basis for antimicrobial stewardship programs, which are the most important local elements in the fight against antibiotic resistance.

In line with this, in the present study, our aim was to analyze the antibiotic resistance properties of ESKAPE pathogens over ten years in the clinical material of our department. We examined the sample types from which these pathogens were most frequently isolated. The incidence of tests with resistant results for each pathogen in aggregate and the most important subgroups of each bacterium was also analyzed. With all these results, we have intended to predict those ESKAPE bacteria that locally most likely will be the main problems.

MATERIALS AND METHODS

Study setting

The present retrospective microbiological study was carried out using data collected, corresponding to period between 1 January 2011 and 31 December 2020, at the Institute of Clinical Microbiology, University of Szeged, Hungary. This clinical microbiology laboratory serves the Albert Szent-Györgyi Clinical Center, which is an 1800-bed primary- and tertiary-care teaching hospital in the Southern Great Plain of Hungary. Data collection was performed electronically, in the records of the laboratory information system, corresponding to clinically relevant samples positive for the ESKAPE pathogens.

Microbiological data set

This study was conducted using local data that were exported from the clinical microbiology laboratory information system (MedBakter, Asseco Central Europe Ltd.,

Hungary), and was reported into a customized database. Data included the types of specimen, species of isolates, and antimicrobial susceptibility patterns. Antimicrobial susceptibility testing results were determined and interpreted according to the EUCAST breakpoints [7].

Data analysis

The data were collected to determine the number of antibiotic susceptibility tests with 'resistant' results and their percentage of the total number of tests performed on the species in question in a given year. Microsoft Excel 2016 software (Microsoft Corp., Redmond, WA, USA) was used to analyze and plot the results. We have also fitted linear trend lines for both variables between 2011 and 2020. We also used the FORECAST function of this software, which predicts a value based on existing ones along with a linear trend. By using this function, we have predicted the expected resistance data for 2021–2030.

Calculating antibiotic resistance index

We have also aimed to evaluate a cumulative antimicrobial resistance index (ARI) as a possible tool to predict the antimicrobial resistance trend. To calculate the ARI, the model for measuring antibiotic resistance in ESKAPE pathogens used by Mencacci et al. [8] was followed. Briefly, for each antibiotic tested in each ESKAPE microorganisms, a score of 0 for susceptibility, 0.5 for intermediate resistance, or 1 for resistance were assigned, and the ARI was calculated by dividing the sum of these scores by the number of antibiotics tested, giving a maximum score of 1. Thus, an ARI of 0 corresponded to a pan drug-susceptible organism and an ARI of 1 to a pan drug-resistant organism. The values obtained were then summarized by species and used to rank the resistance of ESKAPE pathogens.

RESULTS

Distribution of samples and number of isolates included

The results of 1,268,126 antibiotic susceptibility tests carried out on a total of 70,099 ESKAPE isolates were analyzed. The majority of strains were derived from urine, blood culture, tracheal fluid, vaginal fluid, wound fluid, and abscesses (Fig. 1). It can be seen that the prevalence of each pathogen has increased over the decade, albeit to different degrees (Fig. 2A). The MS Excel SLOPE function was used to rank the species. *K. pneumoniae* had the highest slope value, followed in order by *P. aeruginosa*, *E. faecium*, *S. aureus*, *A. baumannii*, and *Enterobacter* spp. (Fig. 2B).

Incidence of antimicrobial susceptibility tests with resistant results

When the occurrence of antibiotic susceptibility tests with resistant results is plotted chronologically by species, it can
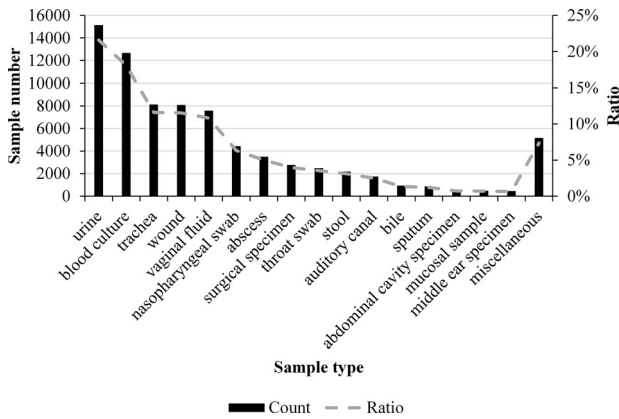
Fig. 1. Distribution of clinical samples containing ESKAPE pathogens by year in the analyzed period (*n* = 70,099 samples). The black bars show the absolute number of samples positive for one of the ESKAPE pathogens, while the dark grey dashed line shows the percentage of positive samples of that sample type within the total number of positive samples

be seen that resistance to any antibiotic was detected most frequently in the cases of *E. faecium* and *A. baumannii* (Fig. 3A). Of particular note is the steep increase in *A. baumannii* at the end of the decade. For the other species, the proportion of resistant tests has been more balanced, but for almost all of them, an increase can be observed in 2020, coinciding with the COVID-19 pandemic (Fig. 3A). Although the full curve slope values do not reflect this phenomenon, the upward trend of *E. faecium* can be seen from the data (Fig. 3B). On this basis, ESKAPE pathogens can be divided into two groups: those with stagnating resistance and those with increasing resistance.

Resistance data per pathogen

In the case of *E. faecium*, the absolute number of resistant tests has steadily increased over the decade, with a slight upward trend in frequency (Fig. 4A). Unfortunately, the prevalence of vancomycin-resistant *E. faecium* (VRE) has increased sharply in both absolute and relative terms
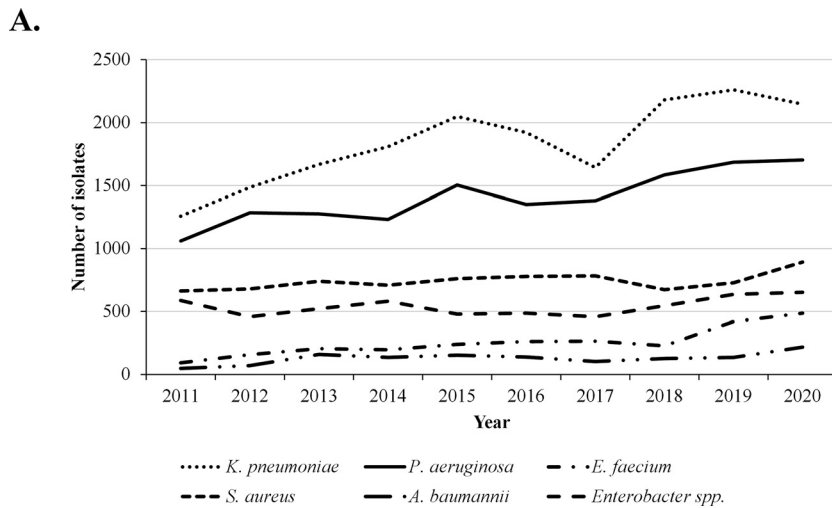
Fig. 2. **A.** Prevalence of ESKAPE pathogens by species and year from 2011 to 2020. **B.** Slope values of the curves describing the prevalence of ESKAPE pathogens
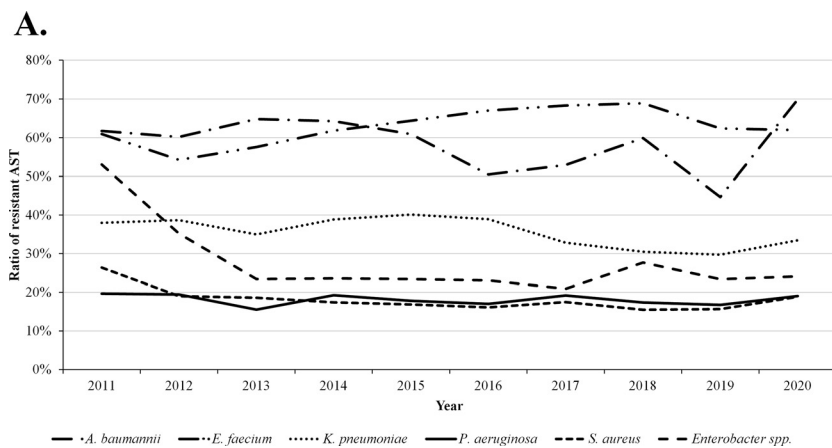
Fig. 3. **A.** Incidence of antimicrobial susceptibility tests with resistant results by species and year from 2011 to 2020. **B.** Slope values of the curves describing the incidence of antimicrobial susceptibility tests with resistant results by species
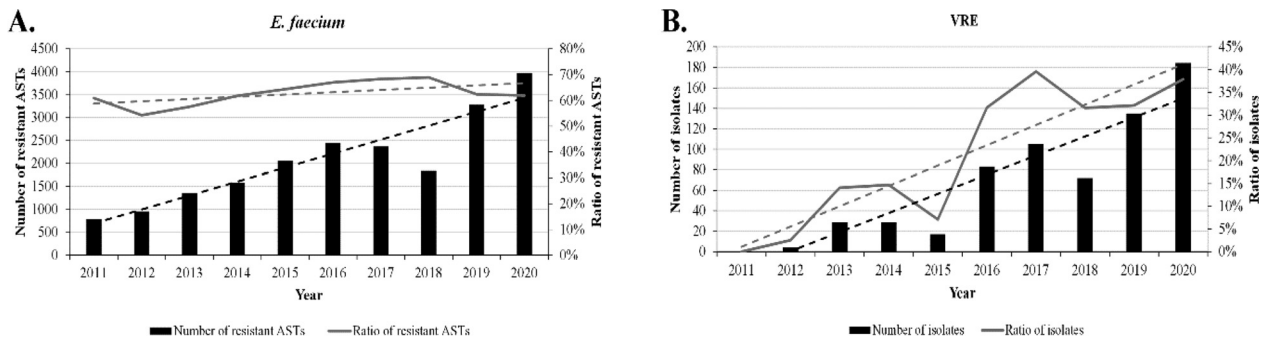
Fig. 4. **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *E. faecium* from 2011 to 2020. **B.** The number and percentage of VRE isolates over the same period. Dashed lines are trend lines of values of the same color

(Fig. 4B). It is worth noting that the increase in the last year of the decade, coincides with the COVID-19 pandemic.

In the case of *S. aureus*, the number of antibiotic susceptibility tests with resistant results has been broadly stable and the incidence has even decreased slightly. However, this relatively favorable trend is expected to break down in 2020 (Fig. 5A).

The tendency is not so favorable for MRSA. A decade-long increase in the absolute number of isolates can be seen, but their relative prevalence has stagnated. Unluckily, the steep increase at the end of the decade is also very pronounced in this case (Fig. 5B).

The absolute number of drug-resistant tests for *K. pneumoniae* has stagnated between 2011 and 2020, and

their relative prevalence shows a slight downward trend. However, this positive trend was broken in the last year of the decade (Fig. 6A).

The absolute number of ESBL-producing *K. pneumoniae* isolates has shown a steady increase over the decade, but their prevalence has decreased. However, a more pronounced increase has also occurred in the last year (Fig. 6B).

In the case of *A. baumannii*, the number of resistant tests increased in absolute terms, but the relative incidence decreased slightly. However, in 2020 a marked increase can be seen (Fig. 7A). The prevalence of carbapenem-resistant *A. baumannii* isolates showed an increase in both absolute and relative terms, finishing with a dramatic increase at the end of the decade (Fig. 7B).
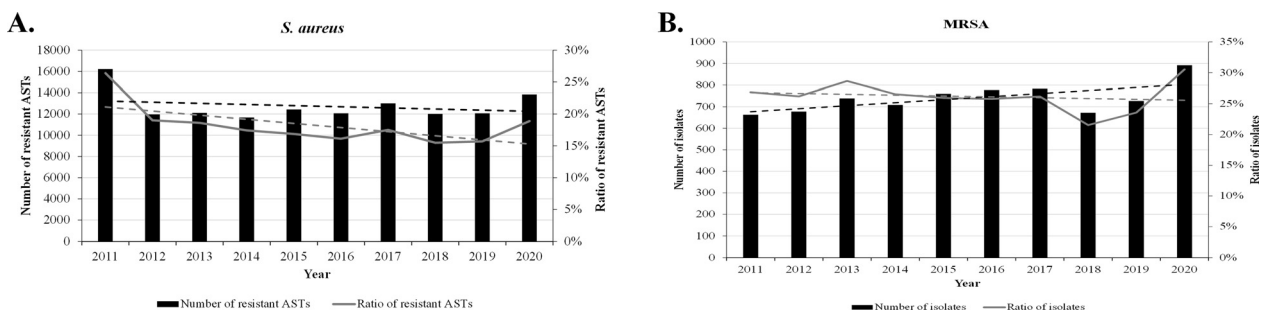

Fig. 5. **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *S. aureus* from 2011 to 2020. **B.** The number and percentage of MRSA isolates over the same period. Dashed lines are trend lines of values of the same color
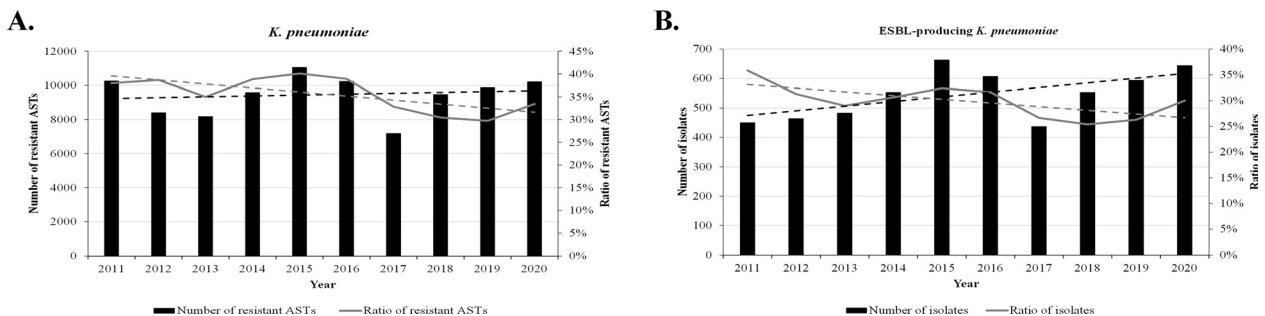

Fig. 6. **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *K. pneumoniae* from 2011 to 2020. **B.** The number and percentage of ESBL-producing *K. pneumoniae* isolates over the same period. Dashed lines are trend lines of values of the same color
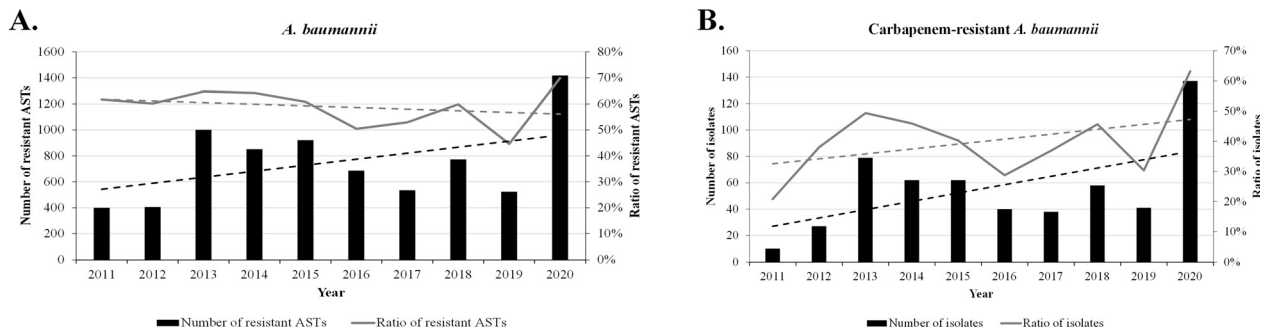
A.

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Fig. 7. **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *A. baumannii* from 2011 to 2020. **B.** The number and percentage of carbapenem-resistant *A. baumannii* isolates over the same period. Dashed lines are trend lines of values of the same color

The absolute number of resistant tests in the case of *P. aeruginosa* has increased over the decade, while their relative frequency has stagnated. Unfortunately, this was replaced by a slight increase in 2020 (Fig. 8A). The Hungarian national recommendation is that all strains of *P. aeruginosa* that are sensitive to only two or less of the listed anti-pseudomonas agents (piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, amikacin) are multi-resistant [9]. The prevalence of multidrug-resistant *P. aeruginosa* has also steadily increased over the period, with a steep rise in 2020 after a peak in 2014 (Fig. 8B).

However, a beneficial trend can be seen for *Enterobacter* species resistant to the tested agents. Both their absolute and relative prevalence have decreased significantly over the decade (Fig. 9A). The same positive trend is noted for the prevalence of ESBL-producing *Enterobacter* species (data not shown). Unfortunately, this trend has been reversed for AmpC-producing species. By 2020, the proportion of AmpC-producing isolates had increased significantly (Fig. 9B).

ARI for ESKAPE pathogens

When calculating the ARI of 70,099 isolates, the most important resistant subgroups (e.g. MRSA or VRE) were managed separately. CRAB, MPAE, ESBL-producing *K. pneumoniae*, VRE, ESBL-producing *Enterobacter* spp., *E. faecium*, and *A. baumannii* had the highest ARI scores based on the 2011–2020 cumulated data (Fig. 10). Our results demonstrate that *A. baumannii*, *P. aeruginosa*, and *E. faecium* and their multi-resistant forms, as well as
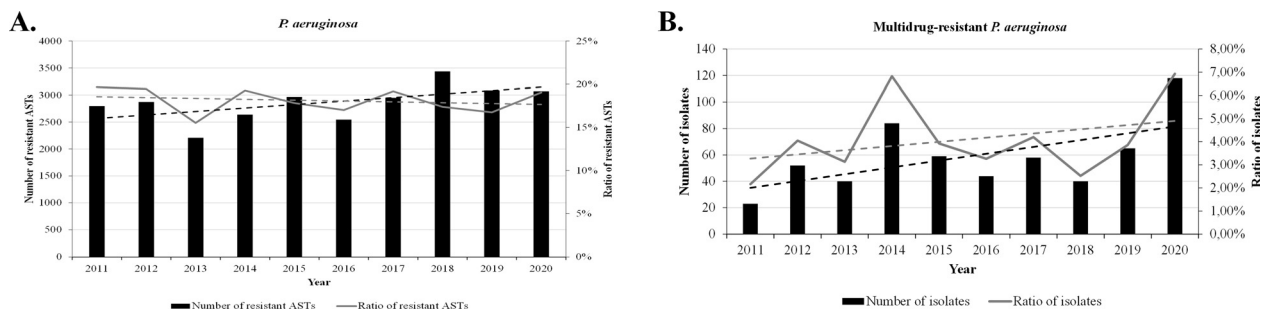
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Fig. 8. **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *P. aeruginosa* from 2011 to 2020. **B.** The number and percentage of multidrug-resistant *P. aeruginosa* isolates over the same period. Dashed lines are trend lines of values of the same color

A.

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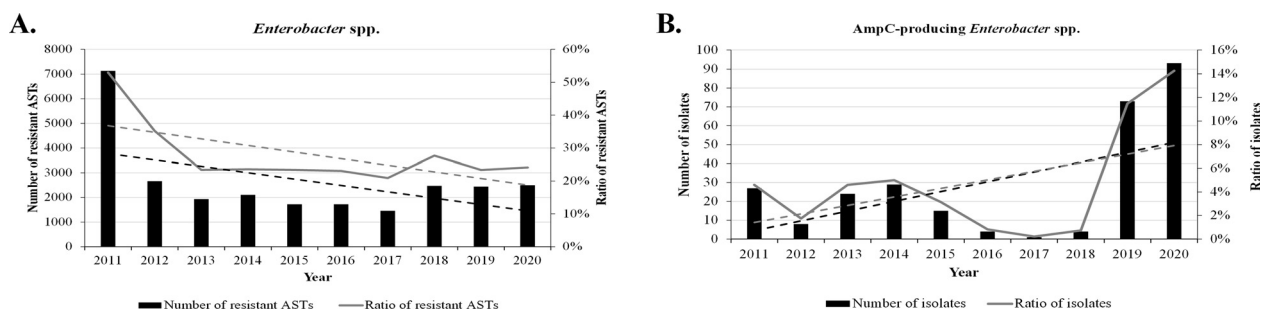*Fig. 9.* **A.** Number and percentage of antibiotic susceptibility tests with resistant results for *Enterobacter* from 2011 to 2020. **B.** The number and percentage of AmpC-producing *Enterobacter* spp. isolates over the same period. Dashed lines are trend lines of values of the same color
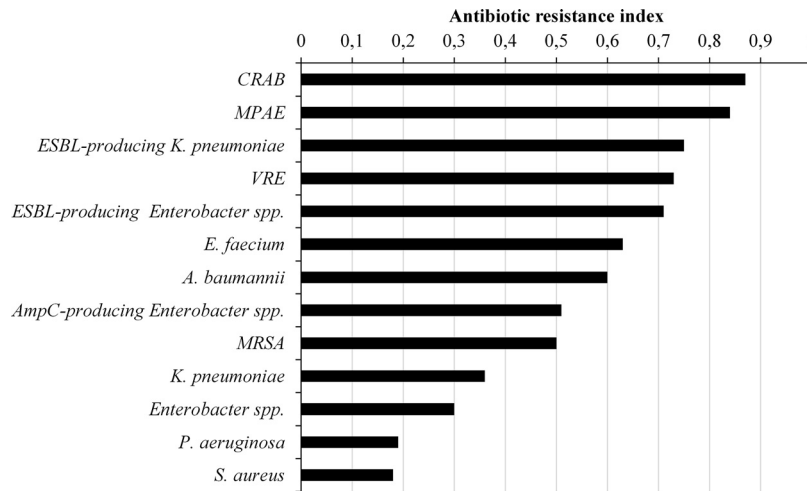
Fig. 10. ARI values in descending order by ESKAPE species and subgroups cumulated from 2011 to 2020

ESBL-producing *K. pneumoniae*, are currently the most challenging ESKAPE pathogens in our care area.

Theoretical prediction of the proportion of resistant antimicrobial susceptibility tests by species for the next ten years

Using the FORECAST function of MS Excel, we attempted to outline trends in antimicrobial resistance over the next 10 years for the ESKAPE species. The development of resistance to these pathogens is expected to be divided into two groups in our area of care.

The first group is composed of members in case of which the proportion of resistant tests is stagnating (Fig. 11). Fortunately, these make up the larger group, members of which include *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *Enterobacter* spp., and their resistant (eg. ESBL-producing) subgroups. The other group, composed of *E. faecium*, *A. baumannii*, and their most problematic resistant sub-categories, is on a path of ever-increasing resistance

(Fig. 11). This trajectory could result in even 86% resistance of antibiotic susceptibility tests performed for VRE by 2030 (Fig. 11).

DISCUSSION

The ESKAPE pathogens are one of the greatest health challenges of our time and have only grown in the shadow of the COVID-19 pandemic [10]. Through genetic mutations and the acquisition of mobile genetic elements, these bacteria have developed resistance mechanisms against different classes of drugs, including those that are the last line of defense, e.g. carbapenems, glycopeptides, and polymyxins [1]. The World Health Organisation has also recognized the importance of this area and has allocated resources for its research [3]. In parallel, local initiatives have been launched to assess the regional ESKAPE pathogen situation and to develop antimicrobial stewardship programs that can respond to this challenge effectively [11–14].
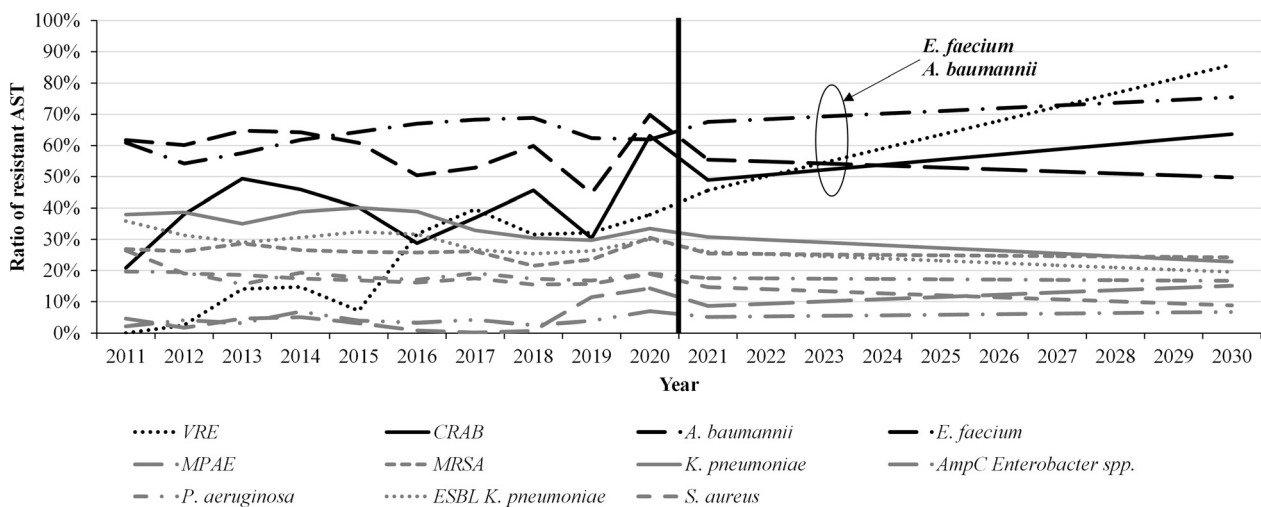

Fig. 11. Theoretical prediction of the proportion of resistant antimicrobial susceptibility tests by species between 2020 and 2030 in our care area (black lines: increasing-resistance group; gray lines: stagnating-resistance group)

In line with this, we have also attempted to explore and predict the ESKAPE problem in our local care area. The distribution of specimens positive for these bacteria has shown the typical landscape of samples positive for nosocomial pathogens (Fig. 1). But the prevalence of all these bacteria increased between 2011 and 2020. The steepest intensifications were seen in the cases of *K. pneumoniae* and *P. aeruginosa* (Fig. 2). The number of antibiotic susceptibility tests with resistant results has also increased over the decade. This was most notable for *E. faecium* and *A. baumannii* (Fig. 3). Resistance trends for each ESKAPE pathogen were quite variable between 2010 and 2020 (Figs 4–9). The definition of ARI values helped us to clarify the situation and establish a ranking (Fig. 10). This was, of course, in good accordance with the number of resistant tests previously defined (Fig. 3). Based on this ranking by the ARI definition, the most serious challenges for us at present are *A. baumannii*, *P. aeruginosa*, and *E. faecium* and their multi-resistant forms. Although this varies by geographical area [11–14], the COVID-19 pandemic has resulted in similar trends worldwide [10, 15–17].

In line with these trends worldwide, the number of VRE and CRAB isolates has increased in the last year of the decade, in parallel with the COVID-19 pandemic (Figs 4B and 7B). This has been accompanied by an increase in the proportion of resistant tests (Fig. 3). Unfortunately, the COVID-19 pandemic has not helped antimicrobial stewardship programs although these are needed now more than ever [18]. According to the literature, the major causes of the increase in antimicrobial resistance in conjunction with the pandemic are mainly related with the rise of empiric antimicrobial use, overcrowding of the healthcare systems, disappearance of stewardship measures and decrease in the rhythm of laboratories activity on surveillance cultures and diagnostic tests to detect antimicrobial-resistant organisms. Furthermore, the increased number of patients in intensive care, prolonged mechanical ventilation, and suffering from ventilator-associated pneumonia may contribute to the colonization with nosocomial pathogens and to the higher number of resistant isolates [19]. A minor influence on resistance development could be associated with the increase of infection control measures adopted to avoid healthcare personnel contamination with SARS-CoV-2, including hand hygiene, the use of personal protective equipment, and devices to decontaminate air, and surfaces. Early antibiotic prescription has been indicated initially as a protective effect of COVID-19 patients, but this might also have played a role in the positive trend of antimicrobial resistance [10].

The theoretical prediction of the proportion of resistant antimicrobial susceptibility tests by species between 2020 and 2030 in our care area draws attention to a worrying trend in the cases of VRE and CRAB strains (Fig. 11). Unless the situation changes decisively for or against (e.g. new, more effective antibiotics are introduced or another pandemic breaks out), the resistance of *E. faecium* and *A. baumannii* will remain on a steadily increasing path. This could make these two species the most challenging ones for us to manage by 2030. The main limitation of our method is its inflexibility. Other publications use more sophisticated formulas, which unfortunately we are not yet qualified to do [8]. However, in the future, we plan to use advanced methods in these studies. Despite this, it is clear our data indicate that the problem of ESKAPE pathogens is ever-growing. To overcome this, an effective antimicrobial stewardship program needs to be launched.

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